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IBM Technical Disclosure Bulletins

**Term:**

I-sce-I

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30

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<u>DB Name</u>	<u>Query</u>	<u>Hit Count</u>	<u>Set Name</u>
USPT,JPAB,EPAB,DWPI	I-sce-I	5	<u>L5</u>
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USPT,JPAB,EPAB,DWPI	11 and endonuclease	1449	<u>L2</u>
USPT,JPAB,EPAB,DWPI	artificial chromosome	2762	<u>L1</u>

**WEST****Generate Collection****Search Results - Record(s) 1 through 12 of 12 returned.**☐ 1. Document ID: US 5962327 A

L4: Entry 1 of 12

File: USPT

Oct 5, 1999

US-PAT-NO: 5962327

DOCUMENT-IDENTIFIER: US 5962327 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

DATE-ISSUED: October 5, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dujon; Bernard	Gif sur Yvette	N/A	N/A	FRX
Choulika; Andre	Paris	N/A	N/A	FRX
Colleaux; Laurence	Edinburgh	N/A	N/A	GBX
Fairhead; Cecile	Malakoff	N/A	N/A	FRX
Perrin; Arnaud	Paris	N/A	N/A	FRX
Plessis; Anne	Paris	N/A	N/A	FRX
Thierry; Agnes	Paris	N/A	N/A	FRX

US-CL-CURRENT: 435/478; 435/320.1, 536/23.2

## ABSTRACT:

An isolated DNA encoding the enzyme I-SceI is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic animals. The vectors are useful in gene mapping and site-directed insertion of genes.

29 Claims, 32 Drawing figures Exemplary Claim Number: 27  
Number of Drawing Sheets: 24

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 2. Document ID: US 5948678 A

L4: Entry 2 of 12

File: USPT

Sep 7, 1999

US-PAT-NO: 5948678

DOCUMENT-IDENTIFIER: US 5948678 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

DATE-ISSUED: September 7, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dujon; Bernard	Gif sur Yvette	N/A	N/A	FRX
Choulika; Andre	Paris	N/A	N/A	FRX
Perrin; Arnaud	Paris	N/A	N/A	FRX
Nicolas; Jean-Francois	Noisy le Roi	N/A	N/A	FRX

US-CL-CURRENT: 435/354; 435/410, 536/23.1, 536/23.74, 536/24.1

## ABSTRACT:

An isolated DNA encoding the enzyme I-SceI is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic animals. The vectors are useful in gene mapping and site-directed insertion of genes.

14 Claims, 64 Drawing figures Exemplary Claim Number: 5  
Number of Drawing Sheets: 46

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	K00C	Draw Desc	Image
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☐ 3. Document ID: US 5866361 A

L4: Entry 3 of 12

File: USPT

Feb 2, 1999

US-PAT-NO: 5866361

DOCUMENT-IDENTIFIER: US 5866361 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

DATE-ISSUED: February 2, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dujon; Bernard	Gif Sur Yvette	N/A	N/A	FRX
Choulika; Andre	Paris	N/A	N/A	FRX
Perrin; Arnaud	Paris	N/A	N/A	FRX
Nicolas; Jean-Francois	Noisy Le Roi	N/A	N/A	FRX

US-CL-CURRENT: 435/69.1; 435/199, 435/252.3, 435/252.33, 530/350, 530/824, 536/23.2

## ABSTRACT:

An isolated DNA encoding the enzyme I-SceI is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic animals. The vectors are useful in gene mapping and site-directed insertion of genes.

8 Claims, 65 Drawing figures Exemplary Claim Number: 1,6  
Number of Drawing Sheets: 46

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 4. Document ID: US 5792632 A

L4: Entry 4 of 12

File: USPT

Aug 11, 1998

US-PAT-NO: 5792632

DOCUMENT-IDENTIFIER: US 5792632 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

DATE-ISSUED: August 11, 1998

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dujon; Bernard	Gif Sur Yvette	N/A	N/A	FRX
Choulika; Andre	Paris	N/A	N/A	FRX
Perrin; Arnaud	Paris	N/A	N/A	FRX
Nicolas; Jean-Francois	Noisy Le Roi	N/A	N/A	FRX

US-CL-CURRENT: 435/462; 435/320.1, 435/468, 435/483

## ABSTRACT:

An isolated DNA encoding the enzyme I-SceI is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic animals. The vectors are useful in gene mapping and site-directed insertion of genes.

17 Claims, 64 Drawing figures Exemplary Claim Number: 1  
Number of Drawing Sheets: 44

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 5. Document ID: US 5474896 A

L4: Entry 5 of 12

File: USPT

Dec 12, 1995

US-PAT-NO: 5474896  
DOCUMENT-IDENTIFIER: US 5474896 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the  
uses thereof

DATE-ISSUED: December 12, 1995

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Dujon; Bernard	Gif sur Yvette	N/A	N/A	FRX
Choulika; Andre	Paris	N/A	N/A	FRX
Colleaux; Laurence	Edinburgh	N/A	N/A	GB6
Fairhead; Cecile	Malakoff	N/A	N/A	FRX
Perrin; Arnaud	Paris	N/A	N/A	FRX
Plessis; Anne	Paris	N/A	N/A	FRX
Thierry; Agnes	Paris	N/A	N/A	FRX

US-CL-CURRENT: 435/6; 435/320.1

ABSTRACT:

An isolated DNA encoding the enzyme I-SceI is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic animals. The vectors are useful in gene mapping and site-directed insertion of genes.

2 Claims, 38 Drawing figures Exemplary Claim Number: 1  
Number of Drawing Sheets: 22

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMC	Draw Desc	Image
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☐ 6. Document ID: US 5792632 A

L4: Entry 6 of 12

File: EPAB

Aug 11, 1998

PUB-NO: US005792632A  
DOCUMENT-IDENTIFIER: US 5792632 A  
TITLE: Nucleotide sequence encoding the enzyme I-SceI and the  
uses thereof

PUBN-DATE: August 11, 1998

INVENTOR-INFORMATION:

NAME	COUNTRY
DUJON, BERNARD	FR
CHOULIKA, ANDRE	FR
PERRIN, ARNAUD	FR
NICOLAS, JEAN-FRANCOIS	FR

INT-CL (IPC): C12N 15/00; C12N 5/00; C12N 15/09; C12N 15/63  
EUR-CL (EPC): C12N009/22; C12N015/66, C12Q001/68 , C12Q001/68

ABSTRACT:

An isolated DNA encoding the enzyme I-SceI is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic animals. The vectors are useful in gene mapping and site-directed insertion of genes.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWC	Draw Desc	Image
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☐ 7. Document ID: WO 9614408 A2

L4: Entry 7 of 12

File: EPAB

May 17, 1996

PUB-NO: WO009614408A2  
DOCUMENT-IDENTIFIER: WO 9614408 A2  
TITLE: NUCLEOTIDE SEQUENCE ENCODING THE ENZYME I-SCEI AND THE  
USES THEREOF

PUBN-DATE: May 17, 1996

INVENTOR-INFORMATION:

NAME	COUNTRY
CHOULIKA, ANDRE	N/A
PERRIN, ARNAUD	N/A
DUJON, BERNARD	N/A
NICOLAS, JEAN-FRANCOIS	N/A

INT-CL (IPC): C12N 15/11; C12N 5/10; C12N 15/66; A01K 67/027  
EUR-CL (EPC): C12N009/22; C12N015/66

ABSTRACT:

An isolated DNA encoding the enzyme I-SceI is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic animals. The vectors are useful in gene mapping and site directed insertion of genes.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWC	Draw. Desc	Image
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☐ 8. Document ID: US 5474896 A

L4: Entry 8 of 12

File: EPAB

Dec 12, 1995



PUB-NO: US005474896A  
DOCUMENT-IDENTIFIER: US 5474896 A  
TITLE: Nucleotide sequence encoding the enzyme I-SceI and the  
uses thereof

PUBN-DATE: December 12, 1995

INVENTOR-INFORMATION:

NAME	COUNTRY
DUJON, BERNARD	FR
CHOULIKA, ANDRE	FR
COLLEAUX, LAURENCE	GB
FAIRHEAD, CECILE	FR
PERRIN, ARNAUD	FR
PLESSIS, ANNE	FR
THIERRY, AGNES	FR

INT-CL (IPC): C12Q 1/68; C12N 15/70  
EUR-CL (EPC): C12Q001/68; C12N009/22, C12Q001/68

ABSTRACT:

An isolated DNA encoding the enzyme I-SceI is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic animals. The vectors are useful in gene mapping and site-directed insertion of genes.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMC	Draw Desc	Image
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☐ 9. Document ID: US 5962327 A

L4: Entry 9 of 12

File: DWPI

Oct 5, 1999

DERWENT-ACC-NO: 1999-589718  
DERWENT-WEEK: 199950  
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TITLE: Nucleotide sequence encoding restriction endonuclease  
I-SecI

INVENTOR: CHOULIKA, A; COLLEAUX, L ; DUJON, B; FAIRHEAD, C ;  
PERRIN, A ; PLESSIS, A ; THIERRY, A

PRIORITY-DATA:

1992US-0971160	November 5, 1992
1992US-0879689	May 5, 1992
1995US-0417226	April 5, 1995

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 5962327 A	October 5, 1999	N/A	051	C12N015/55

INT-CL (IPC): C12N 15/55

ABSTRACTED-PUB-NO: US 5962327A  
BASIC-ABSTRACT:

NOVELTY - Isolated DNA (I) encodes the restriction enzyme I-SceI.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for:

- (1) a DNA (II) comprising (I) operatively linked to a promoter;
- (2) isolated RNA sequence complementary to (I) and RNA complementary to (II);
- (3) a vehicle comprising a vector containing (I);
- (4) a process for the expression of a (I) in a host which is a bacteria or eukaryotic cell;
- (5) a process for the production of I-SceI comprising expressing a vector containing (I) in a recombinant host which is a bacteria or eukaryotic cell; and
- (6) a method for in vivo site directed genetic recombination in an organism using enzyme I-SceI comprises providing an expression vector comprising a synthetic gene encoding I-SceI endonuclease, providing a plasmid comprising an I-SceI restriction site next to or within a gene of interest carried on the plasmid, cotransforming the cells of the organism with the expression vector and the plasmid and then cleaving the I-SceI restriction site which promotes the insertion of the gene of interest into a chromosome of the organism at a specific site by homologous recombination.

USE - (I) can be used as a probe to detect a nucleotide sequence in a biological material such as tissue or body fluids. The probe is labeled with an atom or inorganic radical using a radionuclide or a heavy metal and preferably attached to a water insoluble solid porous support such as nitrocellulose paper.

Purified I-SecI can be used in nested chromosomal fragmentation strategy to genetically map a eukaryotic genome. (I) can be used to generate transgenic organisms containing at least one I-SceI restriction site inserted into a chromosome of the organism. This allows I-SceI to be used for genetic mapping of eukaryotic genomes and in gene therapy to provide a method of replacing a natural gene with another gene that is capable of alleviating a disease or genetic disorder.

ADVANTAGE - Due to the absence of the 18 bp long recognition site for I-SecI restriction endonuclease in the genome of most eukaryotes purified I-SecI can be used in nested chromosomal

fragmentation strategy to genetically map a eukaryotic genome.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMC	Draw Desc	Image
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☐ 10. Document ID: WO 9614408 A2, WO 9614408 A3, EP 791058 A1, US 5792632 A, JP 10508478 W, US 5866361 A, US 5948678 A

L4: Entry 10 of 12

File: DWPI

May 17, 1996

DERWENT-ACC-NO: 1996-251758

DERWENT-WEEK: 199950

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TITLE: Induction of site-directed double strand breaks in chromosomal DNA - to induce homologous recombination between the chromosomal and exogenous DNA

INVENTOR: CHOULIKA, A; DULJON, B; NICOLAS, J ; PERRIN, A

PRIORITY-DATA:

1994US-0336241	November 7, 1994
1992US-0879689	May 5, 1992
1992US-0971160	November 5, 1992
1995US-0465273	June 5, 1995
1998US-0119024	July 20, 1998

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9614408 A2	May 17, 1996	E	123	C12N015/11
WO 9614408 A3	August 29, 1996	N/A	000	C12N015/11
EP 791058 A1	August 27, 1997	E	000	C12N015/11
US 5792632 A	August 11, 1998	N/A	000	C12N015/00
JP 10508478 W	August 25, 1998	N/A	124	C12N015/09
US 5866361 A	February 2, 1999	N/A	000	C12N001/21
US 5948678 A	September 7, 1999	N/A	000	C07H021/04

INT-CL (IPC): A01K 67/027; C07H 21/04; C12N 1/19; C12N 1/21; C12N 5/00; C12N 5/04; C12N 5/06; C12N 5/10; C12N 9/14; C12N 9/16; C12N 15/00; C12N 15/09; C12N 15/11; C12N 15/55; C12N 15/63; C12N 15/66; C12P 21/02; C12N 9/16; C12R 1/865; C12N 9/16; C12R 1/91

ABSTRACTED-PUB-NO: US 5792632A

BASIC-ABSTRACT:

A method to induce at least 1 site-directed double strand (ds) break in a cell's DNA comprises: (a) providing cells contg. ds DNA including at least 1 I-SceI restriction site; (b) transfecting the cells with at least a plasmid comprising DNA encoding the I-SceI meganuclease; and (c) selecting cells in which at least 1 ds break has been induced.

USE - The method is useful to induce homologous recombination between a cell's, pref. a stem cell, chromosomal DNA and exogenous DNA, esp. to insert DNA encoding polypeptides (claimed). By transforming stem cells with the DNAs, polypeptides can be expressed in transgenic animals. Cells and transgenic animals contg. an inserted I-SceI site at a predetermined location are useful for screening procedures, e.g. for phenotypes, ligands and drugs, and for very high level reproducible expression of recombinant retroviral vectors if the cell line is a transcomplementing cell line for retrovirus prodn. Transfected cells, e.g. haematopoietic tissue or skin cells, can be used as targets for gene therapy.

ABSTRACTED-PUB-NO:

#### US 5866361A EQUIVALENT-ABSTRACTS:

A method to induce at least 1 site-directed double strand (ds) break in a cell's DNA comprises: (a) providing cells contg. ds DNA including at least 1 I-SceI restriction site; (b) transfecting the cells with at least a plasmid comprising DNA encoding the I-SceI meganuclease; and (c) selecting cells in which at least 1 ds break has been induced.

USE - The method is useful to induce homologous recombination between a cell's, pref. a stem cell, chromosomal DNA and exogenous DNA, esp. to insert DNA encoding polypeptides (claimed). By transforming stem cells with the DNAs, polypeptides can be expressed in transgenic animals. Cells and transgenic animals contg. an inserted I-SceI site at a predetermined location are useful for screening procedures, e.g. for phenotypes, ligands and drugs, and for very high level reproducible expression of recombinant retroviral vectors if the cell line is a transcomplementing cell line for retrovirus prodn. Transfected cells, e.g. haematopoietic tissue or skin cells, can be used as targets for gene therapy.

A method to induce at least 1 site-directed double strand (ds) break in a cell's DNA comprises: (a) providing cells contg. ds DNA including at least 1 I-SceI restriction site; (b) transfecting the cells with at least a plasmid comprising DNA encoding the I-SceI meganuclease; and (c) selecting cells in which at least 1 ds break has been induced.

USE - The method is useful to induce homologous recombination between a cell's, pref. a stem cell, chromosomal DNA and exogenous DNA, esp. to insert DNA encoding polypeptides (claimed). By transforming stem cells with the DNAs, polypeptides can be expressed in transgenic animals. Cells and transgenic animals contg. an inserted I-SceI site at a predetermined location are useful for screening procedures, e.g. for phenotypes, ligands and drugs, and for very high level reproducible expression of recombinant retroviral vectors if the cell line is a transcomplementing cell line for retrovirus prodn. Transfected cells, e.g. haematopoietic tissue or skin cells, can be used as targets for gene therapy.

US 5948678A

A method to induce at least 1 site-directed double strand (ds) break in a cell's DNA comprises: (a) providing cells contg. ds DNA including at least 1 I-SceI restriction site; (b) transfecting the cells with at least a plasmid comprising DNA encoding the I-SceI meganuclease; and (c) selecting cells in which at least 1 ds break has been induced.

USE - The method is useful to induce homologous recombination between a cell's, pref. a stem cell, chromosomal DNA and exogenous DNA, esp. to insert DNA encoding polypeptides (claimed). By transforming stem cells with the DNAs, polypeptides can be expressed in transgenic animals. Cells and transgenic animals contg. an inserted I-SceI site at a predetermined location are useful for screening procedures, e.g. for phenotypes, ligands and drugs, and for very high level reproducible expression of recombinant retroviral vectors if the cell line is a transcomplementing cell line for retrovirus prodn. Transfected cells, e.g. haematopoietic tissue or skin cells, can be used as targets for gene therapy.

WO 9614408A

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	K/MC	Draw Desc	Image
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☐ 11. Document ID: US 5474896 A

L4: Entry 11 of 12

File: DWPI

Dec 12, 1995

DERWENT-ACC-NO: 1996-039506  
DERWENT-WEEK: 199950  
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TITLE: Mapping the yeast genome by insertion of I-SceI sites -  
cleaving with the enzyme then hybridising purified fragments with  
probes from specific cosmid clones

INVENTOR: CHOULIKA, A; COLLEAUX, L ; DUJON, B ; FAIRHEAD, C ;  
PERRIN, A ; PLESSIS, A ; THIERRY, A

## PRIORITY-DATA:

1992US-0971160

November 5, 1992

1992US-0879689

May 5, 1992

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
US 5474896 A	December 12, 1995	N/A	050	C12Q001/68

INT-CL (IPC): C12N 15/70; C12Q 1/68

ABSTRACTED-PUB-NO: US 5474896A

## BASIC-ABSTRACT:

A yeast genome that does not contain a natural restriction site for I-SceI is genetically mapped by: (i) artificially introducing one, or more I-SceI sites into the genome; (ii) cleaving completely with I-SceI, to produce nested chromosomal fragments; (iii) purifying these fragments by pulsed field gel electrophoresis (PFGE), and transferring the fragments to a solid membrane; (iv) hybridising membrane-bound fragments with a labelled probe derived from the cosmid clones pUKG040, or pUKG066; and (v) detecting the hybridised banding patterns, and using these for mapping.

USE - I-SceI may be used for mapping any eukaryotic (not just yeast) genome, and also (not claimed) for in vivo site-directed genetic recombination, e.g. to replace a natural gene with another gene able to alleviate a disease, or genetic disorder. DNA sequences encoding I-SceI can be used as probes.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMC	Draw. Desc	Clip Img	Image
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☐ 12. Document ID: FR 2701928 A1, WO 9419260 A1

L4: Entry 12 of 12

File: DWPI

Sep 2, 1994

DERWENT-ACC-NO: 1994-281752  
DERWENT-WEEK: 199435  
COPYRIGHT 2000 DERWENT INFORMATION LTD

TITLE: Vehicle-carrying container for sea or air transport - has assembly of rails and plates with lifting mechanisms and counter-weights to allow some vehicles to be set above others

INVENTOR: DILION, B; GRUBER, D ; LECOINTRE, ; GRUBER, R D ;  
LECOINTRE, C

## PRIORITY-DATA:

1993FR-0001979

February 22, 1993

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
FR 2701928 A1	September 2, 1994	N/A	011	B65D088/12
WO 9419260 A1	September 1, 1994	N/A	011	B65D090/00

INT-CL (IPC): B60P 3/08; B65D 88/12; B65D 90/00

ABSTRACTED-PUB-NO: FR 2701928A

## BASIC-ABSTRACT:

The rectangular container (1) has doors at one end (2), and incorporates one or more assemblies (4,5) to position one vehicle above the others inside the container. Each assembly comprises two upper horizontal rails (6) on the upper halves of the container side walls, two lower horizontal rails (8) on the lower halves of the side walls, and a rolling unit made from two parallel lengthwise plates (10) connected to upper and lower bars (11,12) which engage with the rails and can be fixed in position.

The rails are U-shaped with sides perpendicular to the container walls, and the bars of the rolling unit have sliders on their ends which engage with the bars. Each adjustable assembly also has a lifting mechanism and a counterweight (18).

USE/ADVANTAGE - For sea or air transport. The maximum possible volume of the container is used for vehicle transport, with convenient loading and unloading.

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw. Desc	Clip Img	Image
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Generate Collection

Term	Documents
DUJON-B.DWPI,EPAB,JPAB,USPT.	4
DUJON-BERNARD.DWPI,EPAB,JPAB,USPT.	8
(DUJON-B   DUJON-BERNARD)!.DWPI,EPAB,JPAB,USPT.	12

☐ Documents, starting with Document: Display Format:



**WEST**[Generate Collection](#)**Search Results - Record(s) 1 through 5 of 5 returned.**☐ 1. Document ID: US 5948678 A

L5: Entry 1 of 5

File: USPT

Sep 7, 1999

US-PAT-NO: 5948678

DOCUMENT-IDENTIFIER: US 5948678 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 2. Document ID: US 5866361 A

L5: Entry 2 of 5

File: USPT

Feb 2, 1999

US-PAT-NO: 5866361

DOCUMENT-IDENTIFIER: US 5866361 A

TITLE: Nucleotide sequence encoding the enzyme I-SceI and the uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 3. Document ID: US 5804197 A

L5: Entry 3 of 5

File: USPT

Sep 8, 1998

US-PAT-NO: 5804197

DOCUMENT-IDENTIFIER: US 5804197 A

TITLE: Recombinant canine herpesviruses

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 4. Document ID: US 5792632 A

L5: Entry 4 of 5

File: USPT

Aug 11, 1998

US-PAT-NO: 5792632  
DOCUMENT-IDENTIFIER: US 5792632 A  
TITLE: Nucleotide sequence encoding the enzyme I-SceI and the  
uses thereof

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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☐ 5. Document ID: US 5753235 A

L5: Entry 5 of 5

File: USPT

May 19, 1998

US-PAT-NO: 5753235  
DOCUMENT-IDENTIFIER: US 5753235 A  
TITLE: Recombinant canine herpesviruses

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWIC	Draw Desc	Image
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Generate Collection

Term	Documents
I-SCE-I.DWPI,EPAB,JPAB,USPT.	5
I-SCE-IS	0
I-SCE-I.USPT,JPAB,EPAB,DWPI.	5

Display

30

Documents, starting with Document:

5

Display Format: TI

Change Format

(FILE 'HOME' ENTERED AT 18:27:38 ON 19 MAY 2000)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONE'  
ENTERED AT 18:28:04 ON 19 MAY 2000

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L1      10258 S ARTIFICIAL CHROMOSOME
L2      2527 S L1 AND (MAMMALIAN OR MAMMAL)
L3      2 S L2 AND I-SCE-I
L4      1 S L2 AND I-SCEI
L5      271 S I-SCEI OR ISCE-I
L6      326 S I-SCEI OR I-SCE-I
L7      175 S L6 AND CHROMOSOME
L8      69 S L7 AND (MAMMALIAN OR MAMMAL)
L9      23 DUP REM L8 (46 DUPLICATES REMOVED)
L10     23 SORT L9 PY
        E DUJON B?/AU
L11     108 S E4
L12     351 S E2
L13      0 S L11 AND L12
L14     21 S L11 AND I-SCEI
L15     16 DUP REM L14 (5 DUPLICATES REMOVED)
L16     16 SORT L15 PY
L17      4 S L16 AND (MAMMALIAN OR MAMMAL)
L18     57 S L6 AND (RECOMBINANT OR ARTIFICIAL)
L19     30 S L18 AND CHROMOSOME
L20     20 S L19 AND (MAMMALIAN OR MAMMAL)
L21      7 DUP REM L20 (13 DUPLICATES REMOVED)
L22      7 SORT L21 PY

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=> d ti so au ab pi l22 1-7

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L22 ANSWER 1 OF 7 MEDLINE
TI  Rapid physical mapping of YAC inserts by random integration of ***I***
-   ***Sce***      ***I*** sites.
SO  HUMAN MOLECULAR GENETICS, (1993 Mar) 2 (3) 265-71.
    Journal code: BRC. ISSN: 0964-6906.
AU  Colleaux L; Rougeulle C; Avner P; Dujon B
AB  We have developed a novel strategy, based on the random insertion by
    homologous recombination of ***artificial*** ***I*** - ***Sce***
    ***I*** sites within ***mammalian*** repetitive DNA sequences,
which
    should greatly facilitate the high resolution physical mapping of large
    DNA fragments cloned in YAC. A set of transgenic yeast strains containing
    appropriately spaced ***I*** - ***Sce*** ***I*** sites within
    the YAC insert defines a series of nested physical intervals against which
    new genes, clones or DNA fragments can be mapped by simple hybridisation.
    Sequential hybridisation using such a series of nested YAC fragments as
    probes can also allow the rapid sorting of phage or cosmid libraries into
    contigs. This approach, which has been applied to a YAC containing a 460
    kb insert from the mouse X ***chromosome***, may also have
    applications for the restriction mapping of large genomic segments,
    mapping of exons and the search for homologous genes.

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L22 ANSWER 2 OF 7 MEDLINE
TI  Chromosomal double-strand break repair in Ku80-deficient cells.
SO  PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF
    AMERICA, (1996 Aug 20) 93 (17) 8929-33.
    Journal code: PV3. ISSN: 0027-8424.
AU  Liang F; Romanienko P J; Weaver D T; Jeggo P A; Jasin M
AB  The x-ray sensitive hamster cell line xrs-6 is deficient in DNA
    double-strand break (DSB) repair and exhibits impaired V(D)J
    recombination. The molecular defect in this line is in the 80-kDa subunit
    of the Ku autoantigen, a protein that binds to DNA ends and recruits the
    DNA-dependent protein kinase to DNA. Using an ***I*** - ***SceI***
    endonuclease expression system, chromosomal DSB repair was examined in

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xrs-6 and parental CHO-K1 cell lines. A DSB in chromosomal DNA increased the yield of \*\*\*recombinants\*\*\* several thousand-fold above background in both the xrs-6 and CHO-K1 cells, with recombinational repair of DSBs occurring in as many as 1 of 100 cells electroporated with the endonuclease expression vector. Thus, recombinational repair of chromosomal DSBs can occur at substantial levels in \*\*\*mammalian\*\*\* cells and it is not grossly affected in our assay by a deficiency of the Ku autoantigen. Rejoining of broken \*\*\*chromosome\*\*\* ends (end-joining) near the site of the DSB was also examined. In contrast to recombinational repair, end-joining was found to be severely impaired in the xrs-6 cells. Thus, the Ku protein appears to play a critical role in only one of the chromosomal DSB repair pathways.

L22 ANSWER 3 OF 7 SCISEARCH COPYRIGHT 2000 ISI (R)

TI A NOVEL TY1-MEDIATED FRAGMENTATION METHOD FOR NATIVE AND  
 \*\*\*ARTIFICIAL\*\*\* YEAST \*\*\*CHROMOSOMES\*\*\* REVEALS THAT THE MOUSE  
 STEEL GENE IS A HOTSPOT FOR TY1 INTEGRATION  
 SO GENETICS, (JUN 1996) Vol. 143, No. 2, pp. 673-683.  
 ISSN: 0016-6731.

AU DALGAARD J Z; BANERJEE M; CURCIO M J (Reprint)

AB We have developed a powerful new tool for the physical analysis of genomes called Ty1-mediated chromosomal fragmentation and have used the method to map 24 retrotransposon insertions into two different mouse-derived yeast \*\*\*artificial\*\*\* \*\*\*chromosomes\*\*\* (YACs). Expression of a plasmid-encoded GAL1:Ty1 fusion element marked with the retrotransposition indicator gene, ade2AI, resulted in a high fraction of cells that sustained a single Ty1 insertion marked with ADE2. Strains in which Ty1ADE2 inserted into a YAC were identified by cosegregation of the ADE2 gene with the URA3-marked YAC. Ty1ADE2 elements also carried a site for the endonuclease I-DmoI, which we demonstrate is not present anywhere in the yeast genome. Consequently, I-DmoI cleaved a single \*\*\*chromosome\*\*\* or YAC at the unique site of Ty1ADE2 insertion, allowing rapid mapping of integration events. Our analyses showed that the frequency of Ty1ADE2 integration into YACs is equivalent to or higher than that expected based on random insertion. Remarkably, the 50-kb transcription unit of the mouse Steel locus was shown to be a highly significant hotspot for Ty1 integration. The accessibility of \*\*\*mammalian\*\*\* transcription units to Ty1 insertion stands in

contrast  
 to that of yeast transcription units.

L22 ANSWER 4 OF 7 MEDLINE

TI Chromosomal double-strand breaks induce gene conversion at high frequency in \*\*\*mammalian\*\*\* cells.  
 SO MOLECULAR AND CELLULAR BIOLOGY, (1997 Nov) 17 (11) 6386-93.  
 Journal code: NGY. ISSN: 0270-7306.

AU Taghian D G; Nickoloff J A

AB Double-strand breaks (DSBs) stimulate chromosomal and extrachromosomal recombination and gene targeting. Transcription also stimulates spontaneous recombination by an unknown mechanism. We used *Saccharomyces cerevisiae* \*\*\*I\*\*\* - \*\*\*SceI\*\*\* to stimulate recombination between neo direct repeats in Chinese hamster ovary (CHO) cell chromosomal DNA. One neo allele was controlled by the dexamethasone-inducible mouse mammary tumor virus promoter and inactivated by an insertion containing an \*\*\*I\*\*\* - \*\*\*SceI\*\*\* site at which DSBs were introduced in vivo. The other neo allele lacked a promoter but carried 12 phenotypically silent single-base mutations that create restriction sites (restriction fragment length polymorphisms). This system allowed us to generate detailed conversion tract spectra for recipient alleles transcribed at high or low levels. Transient in vivo expression of \*\*\*I\*\*\* - \*\*\*SceI\*\*\* increased homologous recombination 2,000- to 10,000-fold, yielding \*\*\*recombinants\*\*\* at frequencies as high as 1%. Strikingly, 97% of these products arose by gene conversion. Most products had short, bidirectional conversion tracts, and in all cases, donor neo alleles (i.e., those not suffering a DSB) remained unchanged, indicating that conversion was fully nonreciprocal. DSBs in exogenous DNA are usually repaired by end joining requiring little or no homology or by nonconservative homologous recombination (single-strand annealing). In

contrast, we show that chromosomal DSBs are efficiently repaired via conservative homologous recombination, principally gene conversion without associated crossing over. For DSB-induced events, similar recombination frequencies and conversion tract spectra were found under conditions of low and high transcription. Thus, transcription does not further stimulate DSB-induced recombination, nor does it appear to affect the mechanism(s) by which DSBs induce gene conversion.

L22 ANSWER 5 OF 7 SCISEARCH COPYRIGHT 2000 ISI (R)

TI Analysis of gene targeting and intrachromosomal homologous recombination stimulated by genomic double-strand breaks in mouse embryonic stem cells  
SO MOLECULAR AND CELLULAR BIOLOGY, (JUL 1998) Vol. 18, No. 7, pp. 4070-4078.  
Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.  
ISSN: 0270-7306.

AU Donoho G; Jasin M; Berg P (Reprint)

AB To investigate the effects of in vivo genomic DNA double-strand breaks on the efficiency and mechanisms of gene targeting in mouse embryonic stem cells, we have used a series of insertion and replacement vectors carrying two, one, or no genomic sites for the rare-cutting endonuclease \*\*\*I\*\*\* - \*\*\*SceI\*\*\*. These vectors were introduced into the hypoxanthine phosphoribosyltransferase (hprt) gene to produce substrates for gene-targeting (plasmid-to-\*\*\*chromosome\*\*\* ) or intrachromosomal (direct repeat) homologous recombination. Recombination at the hprt locus is markedly increased following transfection with an \*\*\*I\*\*\* - \*\*\*SceI\*\*\* expression plasmid and a homologous donor plasmid (if needed). The frequency of gene targeting in clones with an \*\*\*I\*\*\* - \*\*\*SceI\*\*\* site attains a value of 1%, 5,000 fold higher than that in clones with no \*\*\*I\*\*\* - \*\*\*SceI\*\*\* site. The use of silent restriction site polymorphisms indicates that the frequencies with which donor plasmid sequences replace the target chromosomal sequences decrease with distance from the genomic break site. The frequency of intrachromosomal recombination reaches a value of 3.1%, 120-fold higher than background spontaneous recombination. Because palindromic insertions were used as polymorphic markers, a significant number of \*\*\*recombinants\*\*\* exhibit distinct genotypic sectoring among daughter cells from a single clone, suggesting the existence of heteroduplex DNA in the original recombination product.

L22 ANSWER 6 OF 7 MEDLINE

TI Construction of a \*\*\*recombinant\*\*\* adenovirus for efficient delivery of the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* yeast endonuclease to human cells and its application in the in vivo cleavage of \*\*\*chromosomes\*\*\* to expose new potential telomeres.  
SO NUCLEIC ACIDS RESEARCH, (1999 Nov 1) 27 (21) 4276-81.  
Journal code: O8L. ISSN: 0305-1048.

AU Anglana M; Bacchetti S

AB We have constructed a replication-defective adenovirus vector encoding the yeast \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* endonuclease under the control of the murine cytomegalovirus immediate-early gene promoter (AdM Sce I) for efficient delivery of this enzyme to \*\*\*mammalian\*\*\* cells. We present evidence of AdM Sce I-mediated \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* protein expression and cleavage activity in replication-permissive 293 cells, and of cleavage of \*\*\*chromosomes\*\*\* in vivo in both 293 cells and in non-permissive human cells. We have exploited this system for the generation of \*\*\*chromosomes\*\*\* capped by \*\*\*artificial\*\*\* telomeric sequences in cells with integrated plasmids containing telomeric DNA arrays adjacent to an \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* recognition site. The properties of the AdM Sce I virus described here make it a useful tool for studying biological processes involving induction of DNA breaks, recombination and gene targeting in cells grown in culture and in vivo.

L22 ANSWER 7 OF 7 BIOSIS COPYRIGHT 2000 BIOSIS

TI pSURF-2, a modified BAC vector for selective YAC cloning and functional analysis.  
SO Biotechniques, (July, 1999) Vol. 27, No. 1, pp. 164-175.  
ISSN: 0736-6205.

AU Boyd, A. C. (1); Davidson, H.; Stevenson, B.; McLachlan, G.;  
Davidson-Smith, H.; Porteous, D. J.

AB A modified bacterial \*\*\*artificial\*\*\* \*\*\*chromosome\*\*\* (BAC)  
vector, pSURF-2, adapted for the selective subcloning of yeast  
\*\*\*artificial\*\*\* \*\*\*chromosome\*\*\* (YAC) sequences was constructed.  
DH10B-U, a pyrF derivative of the highly transformable E. coli strain  
DH10B was also constructed and used for the detection of Ura+  
\*\*\*recombinants\*\*\* carrying DNA linked to YAC right arms. The vector's  
properties were illustrated in two main ways. (i) An intact 25-kb YAC  
containing a mouse tyrosinase minigene was cloned into pSURF-2.  
Appropriately spliced tyrosinase RNA was detected by reverse transcription  
(RT)-PCR in extracts of cells transiently lipofected with the cloned YAC.  
(ii) Cells expressing human cystic fibrosis transmembrane conductance  
regulator (CFTR) from an integrated pSURF-2 \*\*\*recombinant\*\*\*  
containing a cDNA expression cassette were selected using the  
hygromycin-resistance (HyTK) marker of the vector and characterized by  
RT-PCR and immunoprecipitation. The unique \*\*\*I\*\*\* - \*\*\*SceI\*\*\* site  
and HyTK marker of pSURF-2 are designed to facilitate subsequent  
functional studies of cloned DNA.

(FILE 'HOME' ENTERED AT 18:27:38 ON 19 MAY 2000)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICONF'  
ENTERED AT 18:28:04 ON 19 MAY 2000

L1 10258 S ARTIFICIAL CHROMOSOME  
L2 2527 S L1 AND (MAMMALIAN OR MAMMAL)  
L3 2 S L2 AND I-SCE-I  
L4 1 S L2 AND I-SCEI  
L5 271 S I-SCEI OR ISCE-I  
L6 326 S I-SCEI OR I-SCE-I  
L7 175 S L6 AND CHROMOSOME  
L8 69 S L7 AND (MAMMALIAN OR MAMMAL)  
L9 23 DUP REM L8 (46 DUPLICATES REMOVED)  
L10 23 SORT L9 PY  
E DUJON B?/AU  
L11 108 S E4  
L12 351 S E2  
L13 0 S L11 AND L12  
L14 21 S L11 AND I-SCEI  
L15 16 DUP REM L14 (5 DUPLICATES REMOVED)  
L16 16 SORT L15 PY  
L17 4 S L16 AND (MAMMALIAN OR MAMMAL)

=> d ti so au ab pi l17 1-4

L17 ANSWER 1 OF 4 CAPLUS COPYRIGHT 2000 ACS  
TI Nucleotide sequence encoding yeast enzyme \*\*\*I\*\*\* - \*\*\*SceI\*\*\* and  
its use in inducing homologous recombination in eukaryotic cells and  
protein production in transgenic animals  
SO PCT Int. Appl., 122 pp.  
CODEN: PIXXD2  
IN Choulika, Andre; Perrin, Arnaud; \*\*\*Dujon, Bernard\*\*\* ; Nicolas,  
Jean-Francois  
AB Synthetic DNA encoding the enzyme \*\*\*I\*\*\* - \*\*\*SceI\*\*\* is provided.  
The DNA sequence can be incorporated in cloning and expression vectors,  
transformed cell lines and transgenic animals. The vectors are useful in  
gene mapping and site-directed insertion of genes. A synthetic gene

encoding *Saccharomyces cerevisiae* \*\*\*I\*\*\* - \*\*\*SceI\*\*\* restriction endonuclease was expressed in *Escherichia coli* and yeast. The enzyme was used in genetic mapping of a yeast chromosome, of YAC's, and of cosmid. \*\*\*I\*\*\* - \*\*\*SceI\*\*\* efficiently induced double-stranded breaks in a chromosomal target in \*\*\*mammalian\*\*\* cells and the breaks were repaired using a donor mol. that shares homol. with the regions flanking the break.

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9614408	A2	19960517	WO 1995-EP4351	19951106
WO 9614408	A3	19960829		
W: CA, JP				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
US 5792632	A	19980811	US 1994-336241	19941107
EP 791058	A1	19970827	EP 1995-938418	19951106
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
JP 10508478	T2	19980825	JP 1995-515058	19951106

L17 ANSWER 2 OF 4 CAPLUS COPYRIGHT 2000 ACS

TI The yeast \*\*\*I\*\*\* - \*\*\*SceI\*\*\* meganuclease induces site-directed chromosomal recombination in \*\*\*mammalian\*\*\* cells

SO C. R. Acad. Sci., Ser. III (1994), 317(11), 1013-9

CODEN: CRASEV; ISSN: 0764-4469

AU Choulika, Andre; Perrin, Arnaud; \*\*\*Dujon, Bernard\*\*\* ; Nicolas, Jean-Francois

AB Double-strand breaks in genomic DNA stimulate recombination. Until now it was not possible to induce in vivo site-directed double-strand breaks in a \*\*\*mammalian\*\*\* chromosomal target. In this article the authors describe the use of \*\*\*I\*\*\* - \*\*\*SceI\*\*\* meganuclease, a very rare cutter yeast endonuclease, to induce site-directed double-strand breaks mediated recombination. The results demonstrate the potential of the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* system for chromosome manipulation in \*\*\*mammalian\*\*\* cells.

L17 ANSWER 3 OF 4 CAPLUS COPYRIGHT 2000 ACS

TI Induction of homologous recombination in \*\*\*mammalian\*\*\* chromosomes by using the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* system of *Saccharomyces cerevisiae*

SO Mol. Cell. Biol. (1995), 15(4), 1968-73

CODEN: MCEBD4; ISSN: 0270-7306

AU Choulika, Andre; Perrin, Arnaud; \*\*\*Dujon, Bernard\*\*\* ; Nicolas, Jean-Francois

AB The mitochondrial intron-encoded endonuclease \*\*\*I\*\*\* - \*\*\*SceI\*\*\* of *Saccharomyces cerevisiae* has an 18-bp recognition sequence and, therefore, has a very low probability of cutting DNA, even within large genomes. The authors demonstrate that double-strand breaks can be initiated by the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* endonuclease at a predetd. location in the mouse genome and that the breaks can be repaired with a donor mol. homologous with regions flanking the breaks. This induced homologous recombination is approx. 2 orders of magnitude more frequent than spontaneous homologous recombination and at least 10 times more frequent than random integration near an active promoter. As a consequence of induced homologous recombination, a heterologous novel sequence can be inserted at the site of the break. This recombination can occur at a variety of chromosomal targets in differentiated and multipotential cells. These results demonstrate homologous recombination involving chromosomal DNA by the double-strand break repair mechanism in \*\*\*mammals\*\*\* and show the usefulness of very rare cutter

endonucleases,

such as \*\*\*I\*\*\* - \*\*\*SceI\*\*\* , for designing genome rearrangements.

L17 ANSWER 4 OF 4 CAPLUS COPYRIGHT 2000 ACS

TI Rapid physical mapping of YAC inserts by random integration of I-Sce I sites

SO Hum. Mol. Genet. (1993), 2(3), 265-71

CODEN: HMGEE5; ISSN: 0964-6906

AU Colleaux, Laurence; Rougeulle, Claire; Avner, Philip; \*\*\*Dujon,\*\*\*  
\*\*\* Bernard\*\*\*

AB This report describes a novel strategy, based on the random insertion by

homologous recombination of artificial \*\*\*I\*\*\* - \*\*\*SceI\*\*\* sites within \*\*\*mammalian\*\*\* repetitive DNA sequences, which should greatly facilitate the high resolu. phys. mapping of large DNA fragments cloned in YAC. A set of transgenic yeast strains contg. appropriately spaced \*\*\*I\*\*\* - \*\*\*SceI\*\*\* sites within the YAC insert defines a series of nested phys. intervals against which new genes, clones or DNA fragments can be mapped by simple hybridization. Sequential hybridization using such a series of nested YAC fragments as probes can also allow the rapid sorting of phage or cosmid libraries into contigs. This approach, which has been applied to a YAC contg. a 460 kb insert from the mouse X chromosome, may also have applications for the restriction mapping of large genomic segments, mapping of exons and the search for homologous genes.

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L9 23 DUP REM L8 (46 DUPLICATES REMOVED)  
L10 23 SORT L9 PY

=> d ti so au ab pi l10 1-23

L10 ANSWER 1 OF 23 MEDLINE

TI Rapid physical mapping of YAC inserts by random integration of \*\*\*I\*\*\*  
- \*\*\*Sce\*\*\* \*\*\*I\*\*\* sites.  
SO HUMAN MOLECULAR GENETICS, (1993 Mar) 2 (3) 265-71.  
Journal code: BRC. ISSN: 0964-6906.  
AU Colleaux L; Rougeulle C; Avner P; Dujon B  
AB We have developed a novel strategy, based on the random insertion by  
homologous recombination of artificial \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\*  
sites within \*\*\*mammalian\*\*\* repetitive DNA sequences, which should  
greatly facilitate the high resolution physical mapping of large DNA  
fragments cloned in YAC. A set of transgenic yeast strains containing  
appropriately spaced \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* sites within  
the YAC insert defines a series of nested physical intervals against which  
new genes, clones or DNA fragments can be mapped by simple hybridisation.  
Sequential hybridisation using such a series of nested YAC fragments as  
probes can also allow the rapid sorting of phage or cosmid libraries into  
contigs. This approach, which has been applied to a YAC containing a 460  
kb insert from the mouse X \*\*\*chromosome\*\*\*, may also have  
applications for the restriction mapping of large genomic segments,  
mapping of exons and the search for homologous genes.

L10 ANSWER 2 OF 23 MEDLINE

TI The yeast \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* meganuclease induces  
site-directed chromosomal recombination in \*\*\*mammalian\*\*\* cells.  
SO COMPTES RENDUS DE L ACADEMIE DES SCIENCES. SERIE III, SCIENCES DE LA VIE,  
(1994 Nov) 317 (11) 1013-9.  
Journal code: CAL. ISSN: 0764-4469.  
AU Chouluka A; Perrin A; Dujon B; Nicolas J F  
AB Double-strand breaks in genomic DNA stimulate recombination. Until now it  
was not possible to induce in vivo site-directed double-strand breaks in a  
\*\*\*mammalian\*\*\* chromosomal target. In this article we describe the  
use of \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* meganuclease, a very rare cutter  
yeast endonuclease, to induce site-directed double-strand breaks mediated



recombination. The results demonstrate the potential of the \*\*\*I\*\*\* -  
 \*\*\*Sce\*\*\* \*\*\*I\*\*\* system for \*\*\*chromosome\*\*\* manipulation in  
 \*\*\*mammalian\*\*\* cells.

L10 ANSWER 3 OF 23 MEDLINE

TI Repair of a specific double-strand break generated within a  
 \*\*\*mammalian\*\*\* \*\*\*chromosome\*\*\* by yeast endonuclease \*\*\*I\*\*\*

SO NUCLEIC ACIDS RESEARCH, (1994 Dec 25) 22 (25) 5649-57.  
 Journal code: OBL. ISSN: 0305-1048.

AU Lukacsovich T; Yang D; Waldman A S

AB We established a mouse Ltk- cell line that contains within its genome a  
 herpes simplex virus thymidine kinase gene (tk) that had been disrupted by  
 the insertion of the recognition sequence for yeast endonuclease \*\*\*I\*\*\*  
 - \*\*\*SceI\*\*\* . The artificially introduced 18 bp \*\*\*I\*\*\* -  
 \*\*\*SceI\*\*\* recognition sequence was likely a unique sequence in the  
 genome of the mouse cell line. To assess whether an induced double-strand  
 break (DSB) in the genomic tk gene would be repaired preferentially by  
 gene targeting or non-homologous recombination, we electroporated the  
 mouse cell line with endonuclease \*\*\*I\*\*\* - \*\*\*SceI\*\*\* alone, one of  
 two different gene targeting constructs alone, or with \*\*\*I\*\*\* -  
 \*\*\*SceI\*\*\* in conjunction with each of the two targeting constructs.  
 Each targeting construct was, in principle, capable of correcting the  
 defective genomic tk sequence via homologous recombination. tk+ colonies  
 were recovered following electroporation of cells with \*\*\*I\*\*\* -  
 \*\*\*SceI\*\*\* in the presence or absence of a targeting construct.

Through

the detection of small deletions at the \*\*\*I\*\*\* - \*\*\*SceI\*\*\*  
 recognition sequence in the mouse genome, we present evidence that a  
 specific DSB can be introduced into the genome of a living  
 \*\*\*mammalian\*\*\* cell by yeast endonuclease \*\*\*I\*\*\* - \*\*\*SceI\*\*\* .  
 We further report that a DSB in the genome of a mouse Ltk- cell is  
 repaired preferentially by non-homologous end-joining rather than by  
 targeted homologous recombination with an exogenous donor sequence. The  
 potential utility of this system is discussed.

L10 ANSWER 4 OF 23 MEDLINE

TI Introduction of double-strand breaks into the genome of mouse cells by  
 expression of a rare-cutting endonuclease.

SO MOLECULAR AND CELLULAR BIOLOGY, (1994 Dec) 14 (12) 8096-106.  
 Journal code: NGY. ISSN: 0270-7306.

AU Rouet P; Smih F; Jasin M

AB To maintain genomic integrity, double-strand breaks (DSBs) in chromosomal  
 DNA must be repaired. In \*\*\*mammalian\*\*\* systems, the analysis of the  
 repair of chromosomal DSBs has been limited by the inability to introduce  
 well-defined DSBs in genomic DNA. In this study, we created specific DSBs  
 in mouse \*\*\*chromosomes\*\*\* for the first time, using an expression  
 system for a rare-cutting endonuclease, \*\*\*I\*\*\* - \*\*\*SceI\*\*\* . A  
 genetic assay has been devised to monitor the repair of DSBs, whereby  
 cleavage sites for \*\*\*I\*\*\* - \*\*\*SceI\*\*\* have been integrated into  
 the mouse genome in two tandem neomycin phosphotransferase genes. We find  
 that cleavage of the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* sites is very efficient,  
 with at least 12% of stably transfected cells having at least one cleavage  
 event and, of these, more than 70% have undergone cleavage at both  
 \*\*\*I\*\*\* - \*\*\*SceI\*\*\* sites. Cleavage of both sites in a fraction of  
 clones deletes 3.8 kb of intervening chromosomal sequences. We find that  
 the DSBs are repaired by both homologous and nonhomologous mechanisms.  
 Nonhomologous repair events frequently result in small deletions after  
 rejoining of the two DNA ends. Some of these appear to occur by simple  
 blunt-ended ligation, whereas several others may occur through annealing  
 of short regions of terminal homology. The DSBs are apparently  
 recombinogenic, stimulating gene targeting of a homologous fragment by  
 more than 2 orders of magnitude. Whereas gene-targeted clones are nearly  
 undetectable without endonuclease expression, they represent approximately  
 10% of cells transfected with the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* expression  
 vector. Gene targeted clones are of two major types, those that occur by  
 two-sided homologous recombination with the homologous fragment and those

that occur by one-sided homologous recombination. Our results are expected to impact a number of areas in the study of \*\*\*mammalian\*\*\* genome dynamics, including the analysis of the repair of DSBs and homologous recombination and, potentially, molecular genetic analyses of \*\*\*mammalian\*\*\* genomes.

L10 ANSWER 5 OF 23 MEDLINE

TI Induction of homologous recombination in \*\*\*mammalian\*\*\*  
 \*\*\*chromosomes\*\*\* by using the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* system of  
 Saccharomyces cerevisiae.  
 SO MOLECULAR AND CELLULAR BIOLOGY, (1995 Apr) 15 (4) 1968-73.  
 Journal code: NGY. ISSN: 0270-7306.  
 AU Choulika A; Perrin A; Dujon B; Nicolas J F  
 AB The mitochondrial intron-encoded endonuclease \*\*\*I\*\*\* - \*\*\*SceI\*\*\*  
 of Saccharomyces cerevisiae has an 18-bp recognition sequence and,  
 therefore, has a very low probability of cutting DNA, even within large  
 genomes. We demonstrate that double-strand breaks can be initiated by the  
 \*\*\*I\*\*\* - \*\*\*SceI\*\*\* endonuclease at a predetermined location in the  
 mouse genome and that the breaks can be repaired with a donor molecule  
 homologous regions flanking the breaks. This induced homologous  
 recombination is approximately 2 orders of magnitude more frequent than  
 spontaneous homologous recombination and at least 10 times more frequent  
 than random integration near an active promoter. As a consequence of  
 induced homologous recombination, a heterologous novel sequence can be  
 inserted at the site of the break. This recombination can occur at a  
 variety of chromosomal targets in differentiated and multipotential cells.  
 These results demonstrate homologous recombination involving chromosomal  
 DNA by the double-strand break repair mechanism in \*\*\*mammals\*\*\* and  
 show the usefulness of very rare cutter endonucleases, such as \*\*\*I\*\*\*  
 - \*\*\*SceI\*\*\*, for designing genome rearrangements.

L10 ANSWER 6 OF 23 CANCERLIT

TI Repair of DNA double strand breaks in \*\*\*mammalian\*\*\* cells by  
 homologous recombination and end-joining mechanisms (Meeting abstract).  
 SO J Cell Biochem, (1995). Suppl. 21A, pp. 328.  
 ISSN: 0730-2312.  
 AU Jasin M; Rouet P; Smih F  
 AB To study the repair of DSBs introduced into \*\*\*mammalian\*\*\*  
 chromosomal DNA, we have developed expression vectors for rare-cutting,  
 site-specific endonucleases from S cerevisiae. We used the universal code  
 equivalent of the mitochondrial intron-encoded endonuclease \*\*\*I\*\*\* -  
 \*\*\*Sce\*\*\* \*\*\*I\*\*\* to build the \*\*\*mammalian\*\*\* expression  
 vector, pCMV- \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\*. The \*\*\*I\*\*\* -  
 \*\*\*Sce\*\*\* \*\*\*I\*\*\* sequence was provided by B Dujon, Pasteur  
 Institute. In addition to providing a consensus Kozak sequence for  
 efficient translation, the \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* ORF was  
 modified by fusing sequences encoding a nuclear localization signal and a  
 hemagglutinin epitope tag. Our initial assay for in vivo cutting and  
 enhanced recombination measures extrachromosomal recombination, since this  
 form of recombination is very efficient in \*\*\*mammalian\*\*\* cells and  
 sensitive to DSBs. The assay utilizes RSVCAT plasmid substrates consisting  
 of overlapping chloramphenicol acetyltransferase (CAT) gene fragments  
 transiently transfected into cells. The RSVCAT plasmids were modified by  
 the insertion of a synthetic \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* site at  
 the end of the homology repeats and cotransfections were carried out in  
 COS 1 cells. We observed a substantial increase of CAT activity in  
 cotransfections of pCMV- \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* with CAT  
 substrates containing the \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* site but  
 not with plasmids lacking the site. Southern analysis verified in vivo  
 cleavage, as well as recombination. Constitutive expression of the  
 endonuclease is not toxic to mouse 3T3 cells. These results have been  
 recently published (Rouet P et al, Proc Natl Acad Sci; 91:6064 1994). We  
 have recently observed efficient cutting of introduced chromosomal  
 \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* sites in 3T3 cells and have found

that

they are recombinogenic, stimulating gene targeting two to three orders of  
 magnitude. However, they are also repaired efficiently by end-joining  
 mechanisms (Rouet P et al, Mol Cell Biol, in press). Results of these

studies were presented. It is expected that expression of rare-cutting endonucleases in \*\*\*mammalian\*\*\* cells will provide a powerful tool for the analysis of the repair of chromosomal DSBs, as well as for molecular genetic manipulations, such as \*\*\*chromosome\*\*\* fragmentation and, potentially, gene-targeting.

L10 ANSWER 7 OF 23 MEDLINE

TI Chromosomal double-strand break repair in Ku80-deficient cells.

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1996 Aug 20) 93 (17) 8929-33.  
Journal code: PV3. ISSN: 0027-8424.

AU Liang F; Romanienko P J; Weaver D T; Jeggo P A; Jasin M

AB The x-ray sensitive hamster cell line xrs-6 is deficient in DNA double-strand break (DSB) repair and exhibits impaired V(D)J recombination. The molecular defect in this line is in the 80-kDa subunit of the Ku autoantigen, a protein that binds to DNA ends and recruits the DNA-dependent protein kinase to DNA. Using an \*\*\*I\*\*\* - \*\*\*SceI\*\*\* endonuclease expression system, chromosomal DSB repair was examined in xrs-6 and parental CHO-K1 cell lines. A DSB in chromosomal DNA increased the yield of recombinants several thousand-fold above background in both the xrs-6 and CHO-K1 cells, with recombinational repair of DSBs occurring in as many as 1 of 100 cells electroporated with the endonuclease expression vector. Thus, recombinational repair of chromosomal DSBs can occur at substantial levels in \*\*\*mammalian\*\*\* cells and it is not grossly affected in our assay by a deficiency of the Ku autoantigen. Rejoining of broken \*\*\*chromosome\*\*\* ends (end-joining) near the site of the DSB was also examined. In contrast to recombinational repair, end-joining was found to be severely impaired in the xrs-6 cells. Thus, the Ku protein appears to play a critical role in only one of the chromosomal DSB repair pathways.

L10 ANSWER 8 OF 23 SCISEARCH COPYRIGHT 2000 ISI (R)

TI MAPPING OF NONINVASION TNP HOA MUTATIONS ON THE ESCHERICHIA-COLI O-18-K1-H7  
\*\*\*CHROMOSOME\*\*\*

SO FEMS MICROBIOLOGY LETTERS, (01 NOV 1996) Vol. 144, No. 2-3, pp. 171-176.  
ISSN: 0378-1097.

AU BLOCH C A (Reprint); HUANG S H; RODE C K; KIM K S

AB The most virulent newborn meningitis-associated Escherichia coli are of the serotype O18:K1:H7. We previously isolated a large number of E. coli O18:K1:H7 mutants resulting from transposon TnpHoA mutagenesis that fail to invade brain microvascular endothelial cells. We have now determined the locations of 45 independent insertions. Twelve were localized to the 98 min region, containing a 120 kb segment that is characteristic of E. coli O18:K1:H7. Another, the previously described insertion ibe-10::TnpHoA, was localized to the 87 min region, containing a 20 kb segment found in this E. coli. These noninvasion mutations may define new O18:K1:H7 pathogenicity islands carrying genes for penetration of the blood-brain barrier of newborn \*\*\*mammals\*\*\*.

L10 ANSWER 9 OF 23 SCISEARCH COPYRIGHT 2000 ISI (R)

TI A NOVEL TY1-MEDIATED FRAGMENTATION METHOD FOR NATIVE AND ARTIFICIAL YEAST  
\*\*\*CHROMOSOMES\*\*\* REVEALS THAT THE MOUSE STEEL GENE IS A HOTSPOT FOR

TY1  
INTEGRATION

SO GENETICS, (JUN 1996) Vol. 143, No. 2, pp. 673-683.  
ISSN: 0016-6731.

AU DALGAARD J Z; BANERJEE M; CURCIO M J (Reprint)

AB We have developed a powerful new tool for the physical analysis of genomes called Ty1-mediated chromosomal fragmentation and have used the method to map 24 retrotransposon insertions into two different mouse-derived yeast artificial \*\*\*chromosomes\*\*\* (YACs). Expression of a plasmid-encoded GAL1:Ty1 fusion element marked with the retrotransposition indicator gene, ade2AI, resulted in a high fraction of cells that sustained a single Ty1 insertion marked with ADE2. Strains in which Ty1ADE2 inserted into a YAC were identified by cosegregation of the ADE2 gene with the URA3-marked YAC. Ty1ADE2 elements also carried a site for the endonuclease I-DmoI, which we demonstrate is not present anywhere in the yeast genome. Consequently, I-DmoI cleaved a single

\*\*\*chromosome\*\*\* or YAC at the unique site of TylADE2 insertion, allowing rapid mapping of integration events. Our analyses showed that the frequency of TylADE2 integration into YACs is equivalent to or higher than that expected based on random insertion. Remarkably, the 50-kb transcription unit of the mouse Steel locus was shown to be a highly significant hotspot for Tyl integration. The accessibility of \*\*\*mammalian\*\*\* transcription units to Tyl insertion stands in contrast to that of yeast transcription units.

L10 ANSWER 10 OF 23 CAPLUS COPYRIGHT 2000 ACS

TI Nucleotide sequence encoding yeast enzyme \*\*\*I\*\*\* - \*\*\*SceI\*\*\* and its use in inducing homologous recombination in eukaryotic cells and protein production in transgenic animals  
 SO PCT Int. Appl., 122 pp.  
 CODEN: PIXXD2  
 IN Choulika, Andre; Perrin, Arnaud; Dujon, Bernard; Nicolas, Jean-Francois  
 AB Synthetic DNA encoding the enzyme \*\*\*I\*\*\* - \*\*\*SceI\*\*\* is provided. The DNA sequence can be incorporated in cloning and expression vectors, transformed cell lines and transgenic animals. The vectors are useful in gene mapping and site-directed insertion of genes. A synthetic gene encoding *Saccharomyces cerevisiae* \*\*\*I\*\*\* - \*\*\*SceI\*\*\* restriction endonuclease was expressed in *Escherichia coli* and yeast. The enzyme was used in genetic mapping of a yeast \*\*\*chromosome\*\*\*, of YAC's, and of cosmid. \*\*\*I\*\*\* - \*\*\*SceI\*\*\* efficiently induced double-stranded breaks in a chromosomal target in \*\*\*mammalian\*\*\* cells and the breaks were repaired using a donor mol. that shares homol. with the regions flanking the break.

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9614408	A2	19960517	WO 1995-EP4351	19951106
	WO 9614408	A3	19960829		
	W: CA, JP				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				
	US 5792632	A	19980811	US 1994-336241	19941107
	EP 791058	A1	19970827	EP 1995-938418	19951106
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE				
	JP 10508478	T2	19980825	JP 1995-515058	19951106

L10 ANSWER 11 OF 23 CAPLUS COPYRIGHT 2000 ACS

TI Stimulation of intrachromosomal homologous recombination in human cells by electroporation with site-specific endonucleases  
 SO Proc. Natl. Acad. Sci. U. S. A. (1996), 93(8), 3608-12  
 CODEN: PNASA6; ISSN: 0027-8424  
 AU Brenneman, Mark; Gimble, Frederick S.; Wilson, John H.  
 AB In somatic \*\*\*mammalian\*\*\* cells, homologous recombination is a rare event. To study the effects of chromosomal breaks on frequency of homologous recombination, site-specific endonucleases were introduced into human cells by electroporation. Cell lines with a partial duplication within the HPRT (hypoxanthine phosphoribosyltransferase) gene were created through gene targeting. Homologous, intrachromosomal recombination between the repeated regions of the gene can reconstruct a functioning, wild-type gene. Treatment of these cells with the restriction endonuclease Xba I, which has a recognition site within the repeated region of HPRT homol., increased the frequency of homologous recombination by more than 10-fold. Recombination frequency was similarly increased by treatment with the rare-cutting yeast endonuclease PI-Sce I when a cleavage site was placed within the repeated of HPRT. In contrast, four restriction enzymes that cut at positions either outside of the repeated regions or between them produced no change in recombination frequency. The results suggest that homologous recombination between intrachromosomal repeats can be specifically initiated by a double-strand break occurring within regions of homol., consistent with the predictions of a double-strand-break-repair model.

L10 ANSWER 12 OF 23 MEDLINE

TI Chromosomal double-strand breaks induce gene conversion at high frequency in \*\*\*mammalian\*\*\* cells.

SO MOLECULAR AND CELLULAR BIOLOGY, (1997 Nov) 17 (11) 6386-93.  
Journal code: NGY. ISSN: 0270-7306.

AU Taghian D G; Nickoloff J A

AB Double-strand breaks (DSBs) stimulate chromosomal and extrachromosomal recombination and gene targeting. Transcription also stimulates spontaneous recombination by an unknown mechanism. We used *Saccharomyces cerevisiae* \*\*\*I\*\*\* - \*\*\*SceI\*\*\* to stimulate recombination between neo direct repeats in Chinese hamster ovary (CHO) cell chromosomal DNA. One neo allele was controlled by the dexamethasone-inducible mouse mammary tumor virus promoter and inactivated by an insertion containing an \*\*\*I\*\*\* - \*\*\*SceI\*\*\* site at which DSBs were introduced in vivo. The other neo allele lacked a promoter but carried 12 phenotypically silent single-base mutations that create restriction sites (restriction fragment length polymorphisms). This system allowed us to generate detailed conversion tract spectra for recipient alleles transcribed at high or low levels. Transient in vivo expression of \*\*\*I\*\*\* - \*\*\*SceI\*\*\* increased homologous recombination 2,000- to 10,000-fold, yielding recombinants at frequencies as high as 1%. Strikingly, 97% of these products arose by gene conversion. Most products had short, bidirectional conversion tracts, and in all cases, donor neo alleles (i.e., those not suffering a DSB) remained unchanged, indicating that conversion was fully nonreciprocal. DSBs in exogenous DNA are usually repaired by end joining requiring little or no homology or by nonconservative homologous recombination (single-strand annealing). In contrast, we show that chromosomal DSBs are efficiently repaired via conservative homologous recombination, principally gene conversion without associated crossing over. For DSB-induced events, similar recombination frequencies and conversion tract spectra were found under conditions of low and high transcription. Thus, transcription does not further stimulate DSB-induced recombination, nor does it appear to affect the mechanism(s) by which DSBs induce gene conversion.

L10 ANSWER 13 OF 23 MEDLINE

TI Repair of site-specific double-strand breaks in a \*\*\*mammalian\*\*\*  
\*\*\*chromosome\*\*\* by homologous and illegitimate recombination.

SO MOLECULAR AND CELLULAR BIOLOGY, (1997 Jan) 17 (1) 267-77.  
Journal code: NGY. ISSN: 0270-7306.

AU Sargent R G; Brenneman M A; Wilson J H

AB In \*\*\*mammalian\*\*\* cells, chromosomal double-strand breaks are efficiently repaired, yet little is known about the relative contributions of homologous recombination and illegitimate recombination in the repair process. In this study, we used a loss-of-function assay to assess the repair of double-strand breaks by homologous and illegitimate recombination. We have used a hamster cell line engineered by gene targeting to contain a tandem duplication of the native adenine phosphoribosyltransferase (APRT) gene with an \*\*\*I\*\*\* - \*\*\*SceI\*\*\* recognition site in the otherwise wild-type APRT+ copy of the gene. Site-specific double-strand breaks were induced by intracellular expression of \*\*\*I\*\*\* - \*\*\*SceI\*\*\*, a rare-cutting endonuclease from the yeast *Saccharomyces cerevisiae*. \*\*\*I\*\*\* - \*\*\*SceI\*\*\* cleavage stimulated homologous recombination about 100-fold; however, illegitimate recombination was stimulated more than 1,000-fold. These results suggest that illegitimate recombination is an important competing pathway with homologous recombination for chromosomal double-strand break repair in \*\*\*mammalian\*\*\* cells.

L10 ANSWER 14 OF 23 MEDLINE

TI \*\*\*I\*\*\* - \*\*\*SceI\*\*\* -induced gene replacement at a natural locus in embryonic stem cells.

SO MOLECULAR AND CELLULAR BIOLOGY, (1998 Mar) 18 (3) 1444-8.  
Journal code: NGY. ISSN: 0270-7306.

AU Cohen-Tannoudji M; Robine S; Choulika A; Pinto D; El Marjou F; Babinet C; Louvard D; Jaisser F

AB Gene targeting is a very powerful tool for studying \*\*\*mammalian\*\*\* development and physiology and for creating models of human diseases. In many instances, however, it is desirable to study different modifications of a target gene, but this is limited by the generally low frequency of homologous recombination in \*\*\*mammalian\*\*\* cells. We have developed a

novel gene-targeting strategy in mouse embryonic stem cells that is based on the induction of endogenous gap repair processes at a defined location within the genome by induction of a double-strand break (DSB) in the gene to be mutated. This strategy was used to knock in an NH2-ezrin mutant in the villin gene, which encodes an actin-binding protein expressed in the brush border of the intestine and the kidney. To induce the DSB, an

\*\*\*I\*\*\* - \*\*\*SceI\*\*\* yeast meganuclease restriction site was first introduced by gene targeting to the villin gene, followed by transient expression of \*\*\*I\*\*\* - \*\*\*SceI\*\*\*. The repair of the ensuing DSB was achieved with high efficiency ( $6 \times 10^{-6}$ ) by a repair shuttle vector sharing only a 2.8-kb region of homology with the villin gene and no negative selection marker. Compared to conventional gene-targeting experiments at the villin locus, this represents a 100-fold stimulation of gene-targeting frequency, notwithstanding a much lower length of homology. This strategy will be very helpful in facilitating the targeted introduction of several types of mutations within a gene of interest.

L10 ANSWER 15 OF 23 MEDLINE

TI Interstitial deletions and intrachromosomal amplification initiated from a double-strand break targeted to a \*\*\*mammalian\*\*\* \*\*\*chromosome\*\*\*

SO EMBO JOURNAL, (1998 Jan 2) 17 (1) 325-33.

Journal code: EMB. ISSN: 0261-4189.

AU Pipiras E; Coquelle A; Bieth A; Debatisse M

AB Interstitial deletions of tumour suppressor genes and amplification of oncogenes are two major manifestations of chromosomal instability in tumour cells. The development of model systems allowing the study of the events triggering these processes is of major clinical importance. Using the properties of the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* nuclease to introduce a localized double-strand break (DSB) in a \*\*\*mammalian\*\*\* \*\*\*chromosome\*\*\* carrying its target sequence, we demonstrate here

that

both types of mutations can be initiated by non-conservative DSB repair pathways. In our system, \*\*\*I\*\*\* - \*\*\*SceI\*\*\* activity dissociates a transfected gpt gene from its promoter, allowing the isolation of gpt-clones. Our results show that intrachromatid single-strand annealing events occur frequently, giving rise to interstitial deletions not accompanied by other chromosomal rearrangements. We also observed that, when present in the cells, extrachromosomal DNA molecules are integrated preferentially at the broken locus. Taking advantage of the insertion of the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* recognition sequence telomeric to and close to the dihydrofolate reductase gene, we show that a less frequent outcome of \*\*\*I\*\*\* - \*\*\*SceI\*\*\* activity is the initiation of cycles of intrachromosomal amplification of this marker, from breaks at a site merging with the enzyme target.

L10 ANSWER 16 OF 23 SCISEARCH COPYRIGHT 2000 ISI (R)

TI Analysis of gene targeting and intrachromosomal homologous recombination stimulated by genomic double-strand breaks in mouse embryonic stem cells

SO MOLECULAR AND CELLULAR BIOLOGY, (JUL 1998) Vol. 18, No. 7, pp. 4070-4078.

Publisher: AMER SOC MICROBIOLOGY, 1325 MASSACHUSETTS AVENUE, NW, WASHINGTON, DC 20005-4171.

ISSN: 0270-7306.

AU Donoho G; Jasin M; Berg P (Reprint)

AB To investigate the effects of in vivo genomic DNA double-strand breaks on the efficiency and mechanisms of gene targeting in mouse embryonic stem cells, we have used a series of insertion and replacement vectors carrying two, one, or no genomic sites for the rare-cutting endonuclease \*\*\*I\*\*\* - \*\*\*SceI\*\*\*. These vectors were introduced into the hypoxanthine phosphoribosyltransferase (hprt) gene to produce substrates for gene-targeting (plasmid-to-\*\*\*chromosome\*\*\* ) or intrachromosomal (direct repeat) homologous recombination. Recombination at the hprt locus is markedly increased following transfection with an \*\*\*I\*\*\* - \*\*\*SceI\*\*\* expression plasmid and a homologous donor plasmid (if needed). The frequency of gene targeting in clones with an \*\*\*I\*\*\* - \*\*\*SceI\*\*\* site attains a value of 1%, 5,000 fold higher than that in clones with no \*\*\*I\*\*\* - \*\*\*SceI\*\*\* site. The use of silent restriction site polymorphisms indicates that the frequencies with which

donor plasmid sequences replace the target chromosomal sequences decrease with distance from the genomic break site. The frequency of intrachromosomal recombination reaches a value of 3.1%, 120-fold higher than background spontaneous recombination. Because palindromic insertions were used as polymorphic markers, a significant number of recombinants exhibit distinct genotypic sectoring among daughter cells from a single clone, suggesting the existence of heteroduplex DNA in the original recombination product.

- L10 ANSWER 17 OF 23 SCISEARCH COPYRIGHT 2000 ISI (R)  
 TI Homology-directed repair is a major double-strand break repair pathway in  
     \*\*\*mammalian\*\*\* cells  
 SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF  
     AMERICA, (28 APR 1998) Vol. 95, No. 9, pp. 5172-5177.  
     Publisher: NATL ACAD SCIENCES, 2101 CONSTITUTION AVE NW, WASHINGTON, DC  
     20418.  
     ISSN: 0027-8424.
- AU Liang F; Han M G; Romanienko P J; Jasin M (Reprint)  
 AB \*\*\*Mammalian\*\*\* cells have been presumed to repair potentially  
     lethal chromosomal double-strand breaks (DSBs) in large part by processes  
     that do not require homology to the break site. This contrasts with  
     *Saccharomyces cerevisiae* where the major DSB repair pathway is homologous  
     recombination. Recently, it has been determined that DSBs in genomic DNA  
     in \*\*\*mammalian\*\*\* cells can stimulate homologous recombination as  
     much as 3 or 4 orders of magnitude, suggesting that homology-directed  
     repair may play an important role in the repair of chromosomal breaks. To  
     determine whether \*\*\*mammalian\*\*\* cells use recombinational repair at  
     a significant level, we have analyzed the spectrum of repair events at a  
     defined chromosomal break by using direct physical analysis of repair  
     products. When an endonuclease-generated DSB is introduced into one of two  
     direct repeats, homologous repair is found to account for 30-50% of  
     observed repair events. Both noncrossover and deletional homologous repair  
     products are detected, at approximately a 1:3 ratio. These results  
     demonstrate the importance of homologous recombination in the repair of  
     DSBs in \*\*\*mammalian\*\*\* cells. In the remaining observed repair  
     events, DSBs are repaired by nonhomologous processes. The nonhomologous  
     repair events generally result in small deletions or insertions at the  
     break site, although a small fraction of events result in larger  
     chromosomal rearrangements. Interestingly, in two insertions, GT repeats  
     were integrated at one of the broken \*\*\*chromosome\*\*\* ends, suggesting  
     that DSB repair can contribute to the spread of microsatellite sequences  
     in \*\*\*mammalian\*\*\* genomes.
- L10 ANSWER 18 OF 23 MEDLINE  
 TI Multiple pathways for repair of DNA double-strand breaks in  
     \*\*\*mammalian\*\*\* \*\*\*chromosomes\*\*\* .  
 SO MOLECULAR AND CELLULAR BIOLOGY, (1999 Dec) 19 (12) 8353-60.  
     Journal code: NGY. ISSN: 0270-7306.
- AU Lin Y; Lukacsovich T; Waldman A S  
 AB To study repair of DNA double-strand breaks (DSBs) in \*\*\*mammalian\*\*\*  
     \*\*\*chromosomes\*\*\* , we designed DNA substrates containing a thymidine  
     kinase (TK) gene disrupted by the 18-bp recognition site for yeast  
     endonuclease \*\*\*I\*\*\* - \*\*\*SceI\*\*\* . Some substrates also contained a  
     second defective TK gene sequence to serve as a genetic donor in  
     recombinational repair. A genomic DSB was induced by introducing  
     endonuclease \*\*\*I\*\*\* - \*\*\*SceI\*\*\* into cells containing a stably  
     integrated DNA substrate. DSB repair was monitored by selection for  
     TK-positive segregants. We observed that intrachromosomal DSB repair is  
     accomplished with nearly equal efficiencies in either the presence or  
     absence of a homologous donor sequence. DSB repair is achieved by  
     nonhomologous end-joining or homologous recombination, but rarely by  
     nonconservative single-strand annealing. Repair of a chromosomal DSB by  
     homologous recombination occurs mainly by gene conversion and appears to  
     require a donor sequence greater than a few hundred base pairs in length.  
     Nonhomologous end-joining events typically involve loss of very few  
     nucleotides, and some events are associated with gene amplification at the  
     repaired locus. Additional studies revealed that precise religation of DNA  
     ends with no other concomitant sequence alteration is a viable mode for

repair of DSBs in a \*\*\*mammalian\*\*\* genome.

L10 ANSWER 19 OF 23 MEDLINE

TI Construction of a recombinant adenovirus for efficient delivery of the  
\*\*\*I\*\*\* - \*\*\*SceI\*\*\* yeast endonuclease to human cells and its  
application in the in vivo cleavage of \*\*\*chromosomes\*\*\* to expose new  
potential telomeres.

SO NUCLEIC ACIDS RESEARCH, (1999 Nov 1) 27 (21) 4276-81.  
Journal code: O8L. ISSN: 0305-1048.

AU Anglana M; Bacchetti S

AB We have constructed a replication-defective adenovirus vector encoding the  
yeast \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\* endonuclease under the  
control of the murine cytomegalovirus immediate-early gene promoter (AdM  
Sce I) for efficient delivery of this enzyme to \*\*\*mammalian\*\*\* cells.  
We present evidence of AdM Sce I-mediated \*\*\*I\*\*\* - \*\*\*Sce\*\*\*  
\*\*\*I\*\*\* protein expression and cleavage activity in replication-  
permissive 293 cells, and of cleavage of \*\*\*chromosomes\*\*\* in vivo in  
both 293 cells and in non-permissive human cells. We have exploited this  
system for the generation of \*\*\*chromosomes\*\*\* capped by artificial  
telomeric sequences in cells with integrated plasmids containing telomeric  
DNA arrays adjacent to an \*\*\*I\*\*\* - \*\*\*Sce\*\*\* \*\*\*I\*\*\*  
recognition site. The properties of the AdM Sce I virus described here  
make it a useful tool for studying biological processes involving  
induction of DNA breaks, recombination and gene targeting in cells grown  
in culture and in vivo.

L10 ANSWER 20 OF 23 MEDLINE

TI \*\*\*Chromosome\*\*\* healing in mouse embryonic stem cells.

SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF  
AMERICA, (1999 Jun 8) 96 (12) 6781-6.  
Journal code: PV3. ISSN: 0027-8424.

AU Sprung C N; Reynolds G E; Jasin M; Murnane J P

AB The addition of new telomeres to the ends of broken \*\*\*chromosomes\*\*\* ,  
termed \*\*\*chromosome\*\*\* healing, has been extensively studied in  
unicellular organisms; however, its role in the \*\*\*mammalian\*\*\* cell  
response to double-strand breaks is unknown. A system for analysis of  
\*\*\*chromosome\*\*\* healing, which involves the integration of plasmid  
sequences immediately adjacent to a telomere, has been established in  
mouse embryonic stem cells. This "marked" telomere contains a neo gene for  
positive selection in G418, an \*\*\*I\*\*\* - \*\*\*SceI\*\*\* endonuclease  
recognition sequence for introducing double-strand breaks, and a herpes  
simplex virus thymidine kinase gene for negative selection with  
ganciclovir for cells that have lost the telomere. Transient expression of  
the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* endonuclease results in terminal deletions  
involving telomeric repeat sequences added directly onto the end of the  
broken \*\*\*chromosome\*\*\* . The sites of addition of the new telomeres  
contain short regions of complementarity to telomeric repeat sequences.  
The most common site of addition is the last A of the ATAA 3' overhang  
generated by the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* endonuclease, without the loss  
of a single nucleotide from the end of the \*\*\*chromosome\*\*\* . The next  
most frequent site involved 5 bp of complementarity, which occurred after  
the loss of four nucleotides from the end of the \*\*\*chromosome\*\*\* . The  
new telomeres are generally much shorter than in the parental cell line,  
and most increase in size with time in culture. These results demonstrate  
that \*\*\*chromosome\*\*\* healing is a mechanism for repair of  
\*\*\*chromosome\*\*\* breaks in \*\*\*mammalian\*\*\* cells.

L10 ANSWER 21 OF 23 BIOSIS COPYRIGHT 2000 BIOSIS

TI pSURF-2, a modified BAC vector for selective YAC cloning and functional  
analysis.

SO Biotechniques, (July, 1999) Vol. 27, No. 1, pp. 164-175.  
ISSN: 0736-6205.

AU Boyd, A. C. (1); Davidson, H.; Stevenson, B.; McLachlan, G.;  
Davidson-Smith, H.; Porteous, D. J.

AB A modified bacterial artificial \*\*\*chromosome\*\*\* (BAC) vector,  
pSURF-2, adapted for the selective subcloning of yeast artificial  
\*\*\*chromosome\*\*\* (YAC) sequences was constructed. DH10B-U, a pyrF  
derivative of the highly transformable E. coli strain DH10B was also



constructed and used for the detection of Ura<sup>+</sup> recombinants carrying DNA linked to YAC right arms. The vector's properties were illustrated in two main ways. (i) An intact 25-kb YAC containing a mouse tyrosinase minigene was cloned into pSURF-2. Appropriately spliced tyrosinase RNA was detected by reverse transcription (RT)-PCR in extracts of cells transiently lipofected with the cloned YAC. (ii) Cells expressing human cystic fibrosis transmembrane conductance regulator (CFTR) from an integrated pSURF-2 recombinant containing a cDNA expression cassette were selected using the hygromycin-resistance (HyTK) marker of the vector and characterized by RT-PCR and immunoprecipitation. The unique \*\*\*I\*\*\* - \*\*\*SceI\*\*\* site and HyTK marker of pSURF-2 are designed to facilitate subsequent functional studies of cloned DNA.

L10 ANSWER 22 OF 23 MEDLINE

TI A double-strand break in a chromosomal LINE element can be repaired by gene conversion with various endogenous LINE elements in mouse cells.

SO MOLECULAR AND CELLULAR BIOLOGY, (2000 Jan) 20 (1) 54-60.

Journal code: NGY. ISSN: 0270-7306.

AU Tremblay A; Jasin M; Chartrand P

AB A double-strand break (DSB) in the \*\*\*mammalian\*\*\* genome has been shown to be a very potent signal for the cell to activate repair processes. Two different types of repair have been identified in \*\*\*mammalian\*\*\* cells. Broken ends can be rejoined with or without

loss

or addition of DNA or, alternatively, a homologous template can be used to repair the break. For most genomic sequences the latter event would involve allelic sequences present on the sister chromatid or homologous \*\*\*chromosome\*\*\*. However, since more than 30% of our genome consists

of

repetitive sequences, these would have the option of using nonallelic sequences for homologous repair. This could have an impact on the evolution of these sequences and of the genome itself. We have designed an assay to look at the repair of DSBs in LINE-1 (L1) elements which number 10(5) copies distributed throughout the genome of all \*\*\*mammals\*\*\*. We introduced into the genome of mouse epithelial cells an L1 element with an \*\*\*I\*\*\* - \*\*\*SceI\*\*\* endonuclease site. We induced DSBs at the \*\*\*I\*\*\* - \*\*\*SceI\*\*\* site and determined their mechanism of repair.

We

found that in over 95% of cases, the DSBs were repaired by an end-joining process. However, in almost 1% of cases, we found strong evidence for repair involving gene conversion with various endogenous L1 elements, with some being used preferentially. In particular, the T(F) family and the L1Md-A2 subfamily, which are the most active in retrotransposition, appeared to be contributing the most in this process. The degree of homology did not seem to be a determining factor in the selection of the endogenous elements used for repair but may be based instead on accessibility. Considering their abundance and dispersion, gene conversion between repetitive elements may be occurring frequently enough to be playing a role in their evolution.

L10 ANSWER 23 OF 23 BIOSIS COPYRIGHT 2000 BIOSIS

TI Production of chromatid breaks by single dsb: Evidence supporting the signal model.

SO International Journal of Radiation Biology., (Jan., 2000) Vol. 76, No. 1, pp. 23-29.

ISSN: 0955-3002.

AU Rogers-Bald, M.; Sargent, R. G.; Bryant, P. E. (1)

AB Purpose: The signal model proposes that all chromatid breaks arise from a single DNA double strand break (dsb) via a recombinational exchange mechanism. Here the prediction that chromatid breaks arise from a single dsb is tested. Method: The genetically engineered Chinese hamster cell line GS19-43 containing a unique yeast \*\*\*I\*\*\* - \*\*\*SceI\*\*\* recognition site was treated with \*\*\*I\*\*\* - \*\*\*SceI\*\*\* endonuclease (Meganuclease) in the presence of the porating agent streptolysin O. Chromatid breaks were scored at 4 h, \*\*\*chromosome\*\*\* breaks at 18 and 22 h following treatment (cells used for a 4 h fixation were prelabelled with BrdU over two cell-cycles). Positive controls were treated with the restriction endonuclease Pst 1. Results: \*\*\*I\*\*\* - \*\*\*SceI\*\*\*

endonuclease produced chromatid breaks and at higher enzyme concentrations isochromatid breaks but no chromatid interchanges. About 16% of the chromatid breaks had a 'colour-switch' between the sister-chromatids at the site of breakage, as revealed by FPG staining. At the longer fixation times (18 and 22 h) \*\*\*chromosome\*\*\* breaks were observed, but again no interchanges were seen. Chromatid and \*\*\*chromosome\*\*\* breaks always appeared on the same \*\*\*chromosome\*\*\*. Conclusions: The production of chromatid breaks from a single dsb fulfils the prediction of the signal model. Moreover, the production of colour-switch breaks at a similar frequency to that for ionizing radiation indicates that chromatid breaks are produced via recombinational exchanges, a significant proportion of which occurs between sister chromatids. The majority is intrachromatid, not involving strand-switches. The absence of interchromosomal exchanges at all fixation times indicates a requirement of two dsb in two different \*\*\*chromosomes\*\*\* for their formation.

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L1: Entry 23 of 24

File: DWPI

May 17, 1996

DERWENT-ACC-NO: 1996-251758

DERWENT-WEEK: 199950

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TITLE: Induction of site-directed double strand breaks in  
chromosomal DNA - to induce homologous recombination between the  
chromosomal and exogenous DNA

INVENTOR: CHOULIKA, A; DUJON, B ; NICOLAS, J ; PERRIN, A

## PRIORITY-DATA:

1994US-0336241	November 7, 1994
1992US-0879689	May 5, 1992
1992US-0971160	November 5, 1992
1995US-0465273	June 5, 1995
1998US-0119024	July 20, 1998

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
WO 9614408 A2	May 17, 1996	E	123	C12N015/11
WO 9614408 A3	August 29, 1996	N/A	000	C12N015/11
EP 791058 A1	August 27, 1997	E	000	C12N015/11
US 5792632 A	August 11, 1998	N/A	000	C12N015/00
JP 10508478 W	August 25, 1998	N/A	124	C12N015/09
US 5866361 A	February 2, 1999	N/A	000	C12N001/21
US 5948678 A	September 7, 1999	N/A	000	C07H021/04

INT-CL (IPC): A01K 67/027; C07H 21/04; C12N 1/19; C12N 1/21; C12N  
5/00; C12N 5/04; C12N 5/06; C12N 5/10; C12N 9/14; C12N 9/16; C12N  
15/00; C12N 15/09; C12N 15/11; C12N 15/55; C12N 15/63; C12N  
15/66; C12P 21/02; C12N 9/16; C12R 1/865; C12N 9/16; C12R 1/91

ABSTRACTED-PUB-NO: US 5792632A

## BASIC-ABSTRACT:

A method to induce at least 1 site-directed double strand (ds)  
break in a cell's DNA comprises: (a) providing cells contg. ds  
DNA including at least 1 I-SceI restriction site; (b)  
transfecting the cells with at least a plasmid comprising DNA  
encoding the I-SceI meganuclease; and (c) selecting cells in  
which at least 1 ds break has been induced.

USE - The method is useful to induce homologous recombination

between a cell's, pref. a stem cell, chromosomal DNA and exogenous DNA, esp. to insert DNA encoding polypeptides (claimed). By transforming stem cells with the DNAs, polypeptides can be expressed in transgenic animals. Cells and transgenic animals contg. an inserted I-SceI site at a predetermined location are useful for screening procedures, e.g. for phenotypes, ligands and drugs, and for very high level reproducible expression of recombinant retroviral vectors if the cell line is a transcomplementing cell line for retrovirus prodn. Transfected cells, e.g. haematopoietic tissue or skin cells, can be used as targets for gene therapy.

ABSTRACTED-PUB-NO:

US 5866361A EQUIVALENT-ABSTRACTS:

A method to induce at least 1 site-directed double strand (ds) break in a cell's DNA comprises: (a) providing cells contg. ds DNA including at least 1 I-SceI restriction site; (b) transfecting the cells with at least a plasmid comprising DNA encoding the I-SceI meganuclease; and (c) selecting cells in which at least 1 ds break has been induced.

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US 5948678A

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WO 9614408A

(FILE 'HOME' ENTERED AT 18:27:38 ON 19 MAY 2000)

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICINF'  
ENTERED AT 18:28:04 ON 19 MAY 2000

L1 10258 S ARTIFICIAL CHROMOSOME  
L2 2527 S L1 AND (MAMMALIAN OR MAMMAL)  
L3 2 S L2 AND I-SCE-I  
L4 1 S L2 AND I-SCEI

=> d ti so au ab pi l3 1-2

L3 ANSWER 1 OF 2 SCISEARCH COPYRIGHT 2000 ISI (R)  
TI A NOVEL TY1-MEDIATED FRAGMENTATION METHOD FOR NATIVE AND ARTIFICIAL YEAST  
CHROMOSOMES REVEALS THAT THE MOUSE STEEL GENE IS A HOTSPOT FOR TY1  
INTEGRATION  
SO GENETICS, (JUN 1996) Vol. 143, No. 2, pp. 673-683.  
ISSN: 0016-6731.  
AU DALGAARD J Z; BANERJEE M; CURCIO M J (Reprint)  
AB We have developed a powerful new tool for the physical analysis of  
genomes called Tyl-mediated chromosomal fragmentation and have used the  
method to map 24 retrotransposon insertions into two different  
mouse-derived yeast artificial chromosomes (YACs).  
Expression of a plasmid-encoded GAL1:Tyl fusion element marked with the  
retrotransposition indicator gene, ade2AI, resulted in a high fraction of  
cells that sustained a single Tyl insertion marked with ADE2. Strains in  
which TylADE2 inserted into a YAC were identified by cosegregation of the  
ADE2 gene with the URA3-marked YAC. TylADE2 elements also carried a site  
for the endonuclease I-DmoI, which we demonstrate is not present anywhere  
in the yeast genome. Consequently, I-DmoI cleaved a single chromosome or  
YAC at the unique site of TylADE2 insertion, allowing rapid mapping of  
integration events. Our analyses showed that the frequency of TylADE2  
integration into YACs is equivalent to or higher than that expected based  
on random insertion. Remarkably, the 50-kb transcription unit of the mouse  
Steel locus was shown to be a highly significant hotspot for Tyl  
integration. The accessibility of mammalian transcription units  
to Tyl insertion stands in contrast to that of yeast transcription units.

L3 ANSWER 2 OF 2 BIOSIS COPYRIGHT 2000 BIOSIS  
TI Rapid physical mapping of YAC inserts by random integration of I  
-Sce I sites.  
SO Human Molecular Genetics, (1993) Vol. 2, No. 3, pp. 265-271.  
ISSN: 0964-6906.  
AU Colleaux, Laurence; Rougeulle, Claire; Avner, Philip; Dujon, Bernard (1)  
AB We have developed a novel strategy, based on the random insertion by  
homeologous recombination of artificial I-Sce  
I sites within mammalian repetitive DNA sequences, which  
should greatly facilitate the high resolution physical mapping of large  
DNA fragments cloned in YAC. A set of transgenic yeast strains containing  
appropriately spaced I-Sce I sites within  
the YAC insert defines a series of nested physical intervals against which  
new genes, clones or DNA fragments can be mapped by simple hybridisation.  
Sequential hybridisation using such a series of nested YAC fragments as  
probes can also allow the rapid sorting of phage or cosmid libraries into  
contigs. This approach, which has been applied to a YAC containing a 460  
kb insert from the mouse X chromosome, may also have applications for the  
restriction mapping of large genomic segments, mapping of exons and the  
search for homologous genes.

FILE 'MEDLINE, AGRICOLA, CANCERLIT, SCISEARCH, CAPLUS, BIOSIS, MEDICINF'  
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L1 10258 S ARTIFICIAL CHROMOSOME  
 L2 2527 S L1 AND (MAMMALIAN OR MAMMAL)  
 L3 2 S L2 AND I-SCE-I  
 L4 1 S L2 AND I-SCEI

=> d ti so au ab pi l4 1

L4 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2000 BIOSIS  
 TI pSURF-2, a modified BAC vector for selective YAC cloning and functional analysis.  
 SO Biotechniques, (July, 1999) Vol. 27, No. 1, pp. 164-175.  
 ISSN: 0736-6205.  
 AU Boyd, A. C. (1); Davidson, H.; Stevenson, B.; McLachlan, G.; Davidson-Smith, H.; Porteous, D. J.  
 AB A modified bacterial artificial chromosome (BAC) vector, pSURF-2, adapted for the selective subcloning of yeast artificial chromosome (YAC) sequences was constructed. DH10B-U, a pyrF derivative of the highly transformable E. coli strain DH10B was also constructed and used for the detection of Ura+ recombinants carrying DNA linked to YAC right arms. The vector's properties were illustrated in two main ways. (i) An intact 25-kb YAC containing a mouse tyrosinase minigene was cloned into pSURF-2. Appropriately spliced tyrosinase RNA was detected by reverse transcription (RT)-PCR in extracts of cells transiently lipofected with the cloned YAC. (ii) Cells expressing human cystic fibrosis transmembrane conductance regulator (CFTR) from an integrated pSURF-2 recombinant containing a cDNA expression cassette were selected using the hygromycin-resistance (HyTK) marker of the vector and characterized by RT-PCR and immunoprecipitation. The unique I-SceI site and HyTK marker of pSURF-2 are designed to facilitate subsequent functional studies of cloned DNA.